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Assessment of the radioprotective effect of propolis in breast cancer patients undergoing radiotherapy. New perspective for an old honey bee product

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ABSTRACT

Background: Ionizing radiation is widely used for treatment of cancer. However, one of the limitations of using radiation is its toxic effects on normal tissue. Radiation damage to normal tissue can be partially reduced by the use of radio-protectors that scavenge free radicals produced during radiation. Recently, interest has increased in the development of potential drug of plant origin for the modification of radiation effects and has an advantage over the synthetic compounds in term of low or no toxicity and with minimum side effects. Propolis is apicultural product which is composed of nutritionally valuable substances and contains considerable amounts of polyphenol substances. Flavonoids and phenolic acids are the major classes of polyphenolic compounds. Because of its broad spectrum biological properties, the interest in propolis as harmless medicine has been increased.

Aim of the work: The present study has been undertaken to evaluate the radio-protective effect of propolis supplementation in breast cancer (BC) patients undergoing radiotherapy.

Subjects and methods: This study included 135 subjects divided into three main groups: Group I: 45 healthy females served as control group of matched age and menopausal status with the next malignant groups. Group II: 45 chemotherapy received breast cancer patients followed by radiation therapy only. Group III: 45 chemotherapy received breast cancer patients followed by radiation therapy plus propolis supplements. Two venous blood samples were collected from both breast cancer patients groups (Before and after radiotherapy) and one blood sample from matched controls. DNA damage in mononuclear cells was assessed by alkaline Comet assay. Serum was separated to measure ribonucleotide reductase M2 subunit (RRM2) by enzyme linked immunossorbent assay (ELISA). Malonaldhyde (MDA), total antioxidant capacity (TAC) and iron were assayed by colorimetric method. One ml blood sample was collected into EDTA tubes for complete blood picture analysis.

Results: The present study showed that radiotherapy is accompanied by significant increase in Comet tail parameters (Tail length, % Tail DNA, Tail moment) in peripheral blood

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mononuclear cells of BC patients. While in the group of patients supplemented with propolis plus radiotherapy, propolis have the ability to reduce significantly the radiation induced DNA damage. Concerning RRM2 subunit, it was found that, although radiotherapy significantly down regulate RRM2 protein but still significantly higher than normal control value. On the other hand, the supplementation of propolis during radiotherapy caused a significant down regulation of RRM2 level and became within the normal control level. Furthermore, radiotherapy is accompanied by significant increase in serum MDA and significant decrease in serum TAC while after propolis supplementation plus radiotherapy, serum MDA and serum TAC significantly improved. Regarding serum iron and hematological parameters including hemoglobin (HB) concentration, white blood cells (WBCs) and platelets counts were significantly decreased after radiotherapy treatment alone while after radiotherapy plus propolis, these parameters significantly increased and became within the normal control level.

Conclusions: Supplementation of propolis with radiotherapy treatment offers a quite measurable protection against DNA damage caused by ionizing radiation in BC patients leukocytes and inhibits RRM2 overexpression. Moreover, propolis has beneficial effects on the serum antioxidant capacity and improves the digestive utilization of iron and the regeneration efficiency of hemoglobin. Larger prospective studies are required to confirm our findings.

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1. Introduction

Radiotherapy is the most common modality for treating human cancers. Eighty percent of cancer patients need radiotherapy at sometime or another, either for curative or palliative purpose (Paul, Unnikrishnan, & Nagappa, 2011). Radiation therapy destroys cells in the exposed area by damaging their genetic material (Sankaranarayanan, 2006). As radiation effects do not discriminate between normal and malignant cells and tissue, patients may experience symptoms during the course of therapy for a few weeks after therapy or months or years later (Karbownik & Reiter, 2000).

Several modalities and clinical approaches have been made to reduce these early and late complications of the radiotherapies and one among them is the use of an effective and non-toxic radio-protector (Kim, Seong, & Youn, 2006). Radioprotectors are compounds that are designed to reduce the damage in normal tissue caused by radiation. These compounds are often antioxidants and must be present before or at the time of radiation for their effectiveness (Maurya, Devasagayam, & Nair, 2006). To overcome harmful effects of synthetic compounds, many naturally occurring substances have been studied as candidates for effective radioprotection. These include polyphenols and honeybee products such as propolis. The awareness of their radio-protective properties has increased over the last decade, and their effects have extensively been studied in vitro and in vivo (Benkovic et al., 2009).

Propolis is adhesive resinous substance manufactured by honey bees from leaf, bud and sap of trees and flower blossoms. Major constituents of propolis are flavonoids, organic acids, phenols, various kinds of enzymes, vitamins and minerals (Bankova, 2005). Because of its broad spectrum of biological properties the interest in propolis as harmless medicine has been increased. Propolis and its active substances flavonoids showed antibacterial, analgesic/anti-inflammatory, antioxidant, immune-enhancement, anti-proliferative activity in cultured human tumor cells and anti-tumor activity in mice. Antioxidant activity of flavonoids is based on ability of direct free radicals scavenging or stabilizing the reactive oxygen species (ROS) by interacting with the reactive compound of the radical (Benković et al., 2008). Flavonoids can also increase the function of the endogenous antioxidant enzyme systems; superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Russo et al., 2000). Moreover, immune activity boosted by propolis and related compounds enhances haemopoietic regeneration and survival following radiation-induced lympho- and myelosupression (Oršolić et al., 2007). Reports of other authors confirm the protective effect of propolis on bone marrow and lymphoid tissue of mice treated with cytotoxic drugs (Lahouel, Boulkour, Segueni, & Fillastre, 2004; Sadzuka, Sugiyama, Shimoi, Kinae, & Hirota, 1997).

Several studies have demonstrated higher initial and/or residual DNA damage and lower repair rate following irradiation of peripheral blood lymphocytes (PBL) in vitro as compared to controls (Palyvoda, Polanska, Wygoda, & Rzeszowska-Wolny, 2003; Lou et al., 2007). Furthermore, many other studies have examined PBL of cancer patients who are undergoing chemotherapy or radiotherapy to see if the effect of radiation or anti-neoplastic drugs results in lymphocyte DNA damage in vivo; this approach is used as a surrogate indicator of how the tumor cells may be affected. These studies have shown that DNA damage is increased and/or DNA repair capacity is decreased in PBL samples from patients with a variety of cancers (Almeida, Duarte, Steward, & Jones, 2006; Nadin, Vargas-Roig, Drago, Ibarra, & Ciocca, 2006).

Among the fundamental processes that influence radiation-induced cell death is DNA repair; a critical molecule in this process is ribonucleotide reductase (RR). RR catalyzes the reduction of ribonucleotides to deoxyribonucleotides and thus provides an essential component for DNA synthesis and repair. RR is composed of two homodimer subunits. The R1 subunit (composed of two molecules of hRRM1) contains the ribonucleotide binding sites and allosteric effector sites. The R2 subunit, which contains a non heme iron complexed with a tyrosyl free radical and is essential for catalytic activity, was initially defined as a homodimer of hRRM2 (Barker et al., 2006). Kuo, Hwang, Sosnay, Kunugi, and Kinsella (2003) showed that the over-expression of the M1 subunit has no effect on radiosensitivity while over-expression of the M2 subunit protects against radiation-induced cell death, consistent with the M2 subunit of RR serving as a potential target for radiosensitization.

The main target of the present study was to evaluate the level of DNA damage in circulating mononuclear cells, ribonucleotide reductase M_2 subunit, total antioxidant capacity, lipid peroxidation as well as hematological patterns in chemotherapy received BC patients before and after completing radiotherapy alone or radiotherapy plus propolis capsules as a radio-protective agent.

2. Subjects and methods

This study included 135 females divided into three main groups:

Group I: 45 healthy females of matched age and menopausal status with the next malignant groups served as control group.
Group II: 45 chemotherapy received breast cancer patients followed by radiation therapy only.
Group III: 45 chemotherapy received breast cancer patients followed by radiation therapy plus propolis supplements.

Patients in group III supplemented with propolis capsules (400 mg, 3 times daily) for 10 consecutive days before radiotherapy, during the course of radiation treatment and 10 days after completing the radiotherapy.

Patients were recruited from Cancer Management and Research Department, Medical Research Institute, Alexandria University. A written consent from all subjects participating in the study was taken according to the declaration of Helsinki and approved by the ethical committee of the Medical Research Institute. All investigations had been carried out in accordance with a high standard of ethics.

All breast cancer patients surgically treated by Modified Radical Mastectomy followed by adjuvant chemotherapy consisting of 6 cycles of FAC (5-Flourouracil, Adriamycin and Cyclophosfamide). After finishing chemotherapy, breast cancer patients exposed to radiation therapy using a linear accelerator (LINAC) which customizes high energy X-rays. The dose delivered was 50 Gray over a period of 25 days in a daily fraction of 2 Gray delivered five times a week. Exclusion criteria including metastatic patients at the time of diagnosis and patients exposed to radiotherapy before surgery.

2.1. Blood samples collection

Two venous blood samples were collected from breast cancer patients (Before and after radiotherapy) and one blood sample from matched controls. About 10 ml fasting venous blood was drawn and divided into two aliquots. The first aliquot was added to heparinized tubes to isolate mononuclear cells by Ficoll-Paque PLUS density gradient centrifugation and processed immediately within a maximum of 1 h period after collection by mean of the alkaline Comet assay to detect DNA damage in peripheral blood mononuclear cells (Cell Biolabs, USA). The second aliquot about 5 ml of venous blood was added to serum separating tube. Blood sample was allowed to clot for 30 min before centrifugation, centrifuged at 3000 rpm for 10 min to isolate serum. The serum was stored at −80 °C until used to assay circulating levels of ribonucleotide reductase R2 subunit (RRM2) by ELISA according to the manufacturer's instructions (Life science, China). Serum Malonaldhyde (MDA), total antioxidant capacity (TAC) and iron were assayed by colorimetric methods. 1 ml blood sample was collected into EDTA tubes for complete blood picture analysis.

2.2. Statistical analysis

Statistical analysis was done using IBM SPSS statistics "version 21". Quantitative data were described by mean as measure of central tendency and standard deviation and minimum, maximum as measure of dispersion. Mixed design ANOVA done to detect statistical significant difference in the mean of quantitative variables before and after therapy and to detect significant interaction among patients who received radiotherapy alone and combined radiotherapy plus propolis supplements. Independent sample t test and Mann Whitney test were used to study statistical significance in the mean of quantitative variables between two patients groups (radiotherapy alone and combined radiotherapy plus propolis supplements). Wilcoxon signed rank test was done to prove statistical significance in the mean of quantitative variables before and after treatment in each group of patients separately. The Kaplan–Meier curve was used to estimate the disease free survival outcome of all patients, and groups were compared with the log-rank statistic. All the statistical tests were two tailed and done at $P < 0.05$ level of significance.

3. Results

3.1. Patient demographics and tumor characteristics

Clinicopathological characteristics of the study patients were represented in Table 1.

3.2. Comet tail parameters

Table 2 showed that, the mean values of Comet tail length in the both chemotherapy received BC patients before treatment either with radiotherapy alone or radiotherapy plus propolis was nearly within the same range (P3 = 0.279) and significantly higher than control value (P1 < 0.001 and <0.001 respectively). The level of this parameter after radiotherapy

Table 1 – Patient demographics and tumor characteristics.

	Chemotherapy received BC patients (n = 90)
Age	
Mean ± SD	53.72 ± 9.92
Range	29–67
Menopausal status	
Pre	36 (40%)
Post	54 (60%)
Histological grade	
II	78 (86.7%)
III	12 (13.3%)
Clinical stage	
II	47 (52.2%)
III	43 (47.8%)
ER status	
Positive	66 (73.3%)
Negative	24 (26.7%)
PR status	
Positive	70 (77.8%)
Negative	20 (22.2%)
Her-2/neu expression	
Positive	30 (33.3%)
Negative	60 (66.7%)
Vascular invasion	
Yes	86 (95.6%)
No	4 (4.4%)
Tumor size (cm)	
≤5	39 (43.3%)
>5	51 (56.7%)
Axillary lymph node involvement	
Positive	68 (75.6%)
Negative	22 (24.4%)

treatment only was significantly increased than its corresponding value before treatment (P2 < 0.001) and still significantly higher than normal control value (P1 < 0.001). On the other hand, after radiotherapy treatment plus propolis, Comet tail length was significantly decreased than the corresponding value before treatment (P2 < 0.001) and became within the normal control level (P1 = 0.316). Moreover the mean values of this parameter after radiotherapy plus propolis was significantly lower than after radiotherapy treatment alone (P3 < 0.001).

Regarding % tail DNA, it was highly elevated before radiotherapy treatment in both chemotherapy received BC patients groups than in control group (P1 < 0.001 and <0.001 respectively). The level of this parameter after radiotherapy treatment only was significantly increased than its corresponding value before treatment (P2 < 0.001) and still significantly higher than normal control value (P1 < 0.001). On the other hand, after radiotherapy treatment plus propolis, % tail DNA was significantly decreased than the corresponding value before treatment (P2 < 0.001) and became within the normal control level (P1 = 0.423). Moreover the mean values of this parameter after radiotherapy plus propolis was significantly lower than after radiotherapy treatment alone (P3 < 0.001).

With respect to Comet tail moment, it was highly elevated before radiotherapy treatment in both chemotherapy received BC patients groups than in control group (P1 < 0.001 and <0.001 respectively). The level of tail moment after radiotherapy treatment only was significantly increased than its corresponding value before treatment (P2 < 0.001) and still significantly higher than normal control value (P1 < 0.001). On the other hand, after radiotherapy treatment plus propolis supplements, Comet tail moment was significantly decreased than the corresponding value before treatment (P2 < 0.001) and became within the normal control level (P1 = 0.521). Moreover, the mean value of this parameter after radiotherapy plus propolis was significantly lower than after radiotherapy treatment alone (P3 < 0.001).

Table 2 – Statistical analyses of Comet tail parameters in normal control subjects and chemotherapy received breast cancer patients before and after treatment with either radiotherapy alone or radiotherapy plus propolis supplements.

	Control group (n = 45)	Chemotherapy received breast cancer patients			
		Radiotherapy alone group (n = 45)		Radiotherapy + propolis group (n = 45)	
		Before	After	Before	After
Tail length (μm)					
Range	13.17–32.71	31.15–47.09	52.3–131.96	31.67–48.82	15.21–34.42
Mean ± SD	20.69 ± 6.01	38.99 ± 4.50	78.55 ± 18.88	40.59 ± 4.9	22.73 ± 3.95
P_1		<0.001*	<0.001*	<0.001*	0.316
P2		<0.001*		<0.001*	
P3				0.279	<0.001*
% Tail DNA					
Range	1.31–8.91	4.25–31.41	24.24–70.50	3.89–26.93	2.21–10.14
Mean ± SD	4.83 ± 2.03	15.19 ± 6.77	39.87 ± 12.27	17.37 ± 6.13	6.14 ± 3.19
P_1		<0.001*	<0.001*	<0.001*	0.423
P2		<0.001*		<0.001*	
P3				0.157	<0.001*
Tail moment					
Range	0.28–2.91	1.59–11.53	12.98–93.09	1.25–10.94	0.53–4.72
Mean ± SD	1.95 ± 0.68	6.90 ± 2.62	32.56 ± 17.20	7.16 ± 2.75	2.29 ± 1.11
P_1		<0.001*	<0.001*	<0.001*	0.521
P2		<0.001*		<0.001*	
P3				0.804	<0.001*

3.3. Serum RRM2, TAC, MDA and iron

Table 3 showed that, the mean values of serum RRM2 subunit in the both chemotherapy received BC patients before treatment either with radiotherapy alone or radiotherapy plus propolis supplements was nearly within the same range (P3 = 0.286) and significantly higher than control values (P1 < 0.001 and <0.001 respectively). After radiotherapy treatment only serum RRM2 was significantly decreased than its corresponding value before treatment (P2 < 0.001) and still significantly higher than normal control value (P1 < 0.001). On the other hand, after radiotherapy treatment plus propolis the level of RRM2 subunit was significantly decreased than the corresponding value before treatment (P2 < 0.001) and became within the normal control level (P1 = 0.563). Moreover the mean values of this enzyme subunit after radiotherapy plus propolis was significantly lower than after radiotherapy treatment alone (P3 < 0.001).

As presented in Table 3, the mean values of TAC in the two chemotherapy received BC patients groups was significantly less than normal control levels (P1 < 0.001 and <0.001 respectively). Serum TAC after radiotherapy treatment alone was significantly decreased than corresponding values before treatment (P2 = 0.013) and was significantly lower than normal control level (P1 < 0.001 and <0.001 respectively). On the other hand, the level of this parameter after radiotherapy plus propolis was significantly increased than its corresponding value before treatment (P2 < 0.001) and still significantly less than normal control value (P1 < 0.001). Moreover TAC levels after radiotherapy plus propolis were significantly higher than after radiotherapy treatment alone (P3 < 0.001).

Regarding serum MDA levels in both chemotherapy received BC patients groups, it was significantly higher than normal control values (P1 < 0.001 and <0.001 respectively). After treatment with radiotherapy alone, serum MDA levels were significantly increased than its corresponding value before treatment (P2 < 0.001) and were significantly higher than normal control levels (P1 < 0.001). On the other hand, after radiotherapy treatment plus propolis, serum MDA was significantly decreased than its corresponding value before treatment (P2 < 0.001) and still significantly higher than normal control value (P1 < 0.001). Moreover, it was observed that serum MDA after radiotherapy plus propolis was significantly lower than after radiotherapy treatment alone (P3 < 0.001).

With respect to serum iron, it was highly decreased in both chemotherapy received BC patients groups than normal control values (P1 < 0.001 and <0.001 respectively). In radiotherapy alone group, serum iron after radiotherapy significantly decreased than corresponding values before treatment (P2 = 0.021) and significantly lower than normal control levels (P1 < 0.001 and <0.001 respectively). On the other hand, after radiotherapy treatment plus propolis the level of serum iron was significantly increased than its corresponding value before treatment (P2 < 0.001) and became within the normal control value (P1 = 0.915). Furthermore, it was noticed that, serum iron after radiotherapy treatment plus propolis supplements was significant higher than after radiotherapy treatment only (P3 < 0.001).

Table 3 – Statistical analyses of serum RRM2, TAC, MDA and iron in normal control subjects and chemotherapy received breast cancer patients before and after treatment with either radiotherapy alone or radiotherapy plus propolis supplements.

	Control group (n = 45)	Chemotherapy received breast cancer patients			
		Radiotherapy alone group (n = 45)		Radiotherapy + propolis group (n = 45)	
		Before	After	Before	After
RRM2 (pg/ml)					
Range	65–300	460–1500	280–710	330–1450	125–400
Mean ± SD	167.48 ± 67.49	714.4 ± 224.7	477.80 ± 114	661.2 ± 268.27	186.4 ± 79.86
P_1		<0.001*	<0.001*	<0.001*	0.563
P2		<0.001*		<0.001*	
P3				0.286	<0.001*
TAC (mM/L)					
Range	0.81–1.81	0.16–0.72	0.19–0.61	0.13–0.64	0.55–1.90
Mean ± SD	1.34 ± 0.26	0.49 ± 0.15	0.30 ± 0.10	0.43 ± 0.14	0.89 ± 0.24
P_1		<0.001*	<0.001*	<0.001*	<0.001*
P2		0.013*		<0.001*	
P3				0.217	<0.001*
MDA (nmol/ml)					
Range	1.01–3.21	5.30–17.30	6.17–30.27	5.81–18.65	2.40–10.57
Mean ± SD	1.59 ± 0.60	11.77 ± 2.97	16.12 ± 6.27	11.37 ± 3.03	6.48 ± 2.32
P_1		<0.001*	<0.001*	<0.001*	<0.001*
P2		<0.001*		<0.001*	
P3				0.503	<0.001*
Iron (μg/dl)					
Range	113.2–186.7	39.2–122.47	34.7–112.17	26.95–92.13	85.2–282.1
Mean ± SD	140.38 ± 47.85	80.57 ± 22.14	65.11 ± 20.73	72.92 ± 17.84	137.8 ± 43.24
P_1		<0.001*	<0.001*	<0.001*	0.915
P2		0.021*		<0.001*	
P3				0.070	<0.001*

Table 4 – Statistical analyses of hematological parameters in normal control subjects and chemotherapy received breast cancer patients before and after treatment with either radiotherapy alone or radiotherapy plus propolis supplements.

	Control group (n = 45)	Chemotherapy received breast cancer patients			
		Radiotherapy alone group (n = 45)		Radiotherapy + propolis group (n = 45)	
		Before	After	Before	After
HB (g/dl)					
Range	11.30–14.60	10.0–14.10	8.10–12.10	9.80–14.20	7.60–14.60
Mean ± SD	12.50 ± 1.13	12.13 ± 1.04	10.21 ± 0.93	11.88 ± 1.12	11.92 ± 1.42
P_1		0.921	<0.001*	0.563	0.681
P2		<0.001*		0.861	
P3				0.081	<0.001*
WBCs (10^9/L)					
Range	4.71–10.60	3.19–9.7	2.10–9.84	3.5–8.68	2.95–10.0
Mean ± SD	6.3 ± 0.84	5.98 ± 1.60	4.01 ± 1.50	6.01 ± 1.66	5.88 ± 1.81
P_1		0.571	<0.001*	0.461	0.380
P2		<0.001*		0.492	
P3				0.180	<0.001*
Platelets (10^9/L)					
Range	270–430	192–378	169–367	159–350	230–410
Mean ± SD	323.3 ± 60.1	307.4 ± 72.5	268.1 ± 54.1	300.6 ± 79.6	355.9 ± 115.3
P_1		0.451	<0.001*	0.145	0.213
P2		<0.001*		<0.001*	
P3				0.574	<0.001*

3.4. *Hematological results*

Table 4 revealed that, the mean values of hemoglobin concentration in the both chemotherapy received BC patients before treatment either with radiotherapy alone or radiotherapy plus propolis was nearly within the same range (P3 = 0.081) and showed insignificant difference with normal control value (P1 = 0.921 and 0.563 respectively). The level of this parameter after radiotherapy treatment only was significantly decreased than its corresponding value before treatment (P2 < 0.001) and still significantly lower than normal control value (P1 < 0.001). On the other hand, after radiotherapy treatment plus propolis, hemoglobin concentration showed insignificant difference with its corresponding value before treatment (P2 = 0.861) and became within the normal control level (P1 = 0.681). Moreover hemoglobin concentration after radiotherapy plus propolis was significantly higher than after radiotherapy treatment alone (P3 < 0.001).

As regards white blood cells (WBCs) counts in both chemotherapy received BC patients groups, it showed insignificant difference with normal control value (P1 = 0.571 and 0.461 respectively). The level of this parameter after radiotherapy treatment only was significantly decreased than its corresponding value before treatment (P2 < 0.001) and still significantly lower than normal control value (P1 < 0.001). On the other hand, after radiotherapy treatment plus propolis, WBCs counts showed insignificant difference with its corresponding value before treatment (P2 = 0.492) and became within the normal control level (P1 = 0.380). Moreover it was noticed that, WBCs count after radiotherapy plus propolis was significantly higher than after radiotherapy treatment alone (P3 < 0.001).

As shown in Table 4, the mean values of platelets counts before radiotherapy treatment in the both chemotherapy received BC patients groups was nearly within the same range (P3 = 0.574) and showed insignificant difference with normal control value (P1 = 0.451 and 0.145 respectively). The level of this parameter after radiotherapy treatment only was significantly decreased than its corresponding value before treatment (P2 < 0.001) and still significantly lower than normal control values (P1 < 0.001). On the other hand, after radiotherapy treatment plus propolis supplements, platelets counts significantly increased than its corresponding value before treatment (P2 < 0.001) and became within the normal control level (P1 = 0.231). Moreover the mean values of platelets counts after radiotherapy plus propolis was significantly higher than after radiotherapy treatment alone (P3 < 0.001).

3.5. *The impact of propolis administration on disease free survival in breast cancer patients*

Fig. 1 shows that, breast cancer patients supplemented with propolis plus radiotherapy had longer median disease free

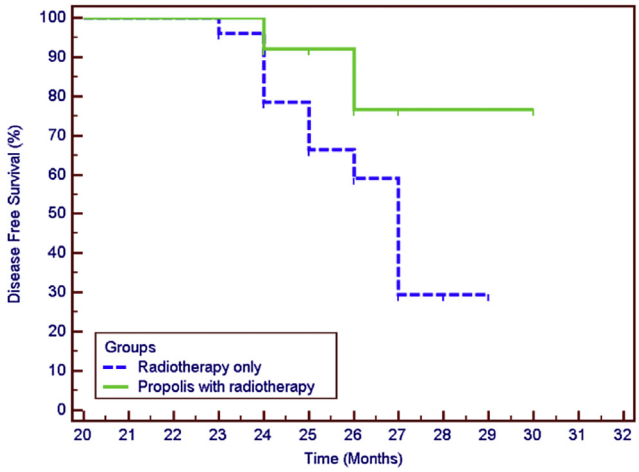

Fig. 1 – Disease free survival according to propolis administration in breast cancer patients.

Table 5 – Test of significance for disease free survival according to propolis administration.

	Median survival (months)	Log rank	
		χ^2	P
Radiotherapy only	23	5.170*	0.023*
Propolis with radiotherapy	27		

survival time (27 months) than patients received radiotherapy alone (23 months). The difference was statistically significant using log rank test (0.023) (Table 5).

4. Discussion

One of the major challenges in cancer research is to discover non-toxic, selective, and effective cytoprotective compounds that would preferentially protect normal tissue during radiotherapy or chemotherapy. These compounds should be effective against genetic damage, mutation and changes to the immune system (Benkovic et al., 2009).

Recently, there has been renewed interest in search for plant derived drug as potential radio-protectors (Hosseinimehr, 2007). Propolis is a product of great interest, both in the field of medicine and the pharmaceutical industry with numerous properties including antioxidant, anti-inflammatory, immune stimulant, hepatoprotector, and carcinostatic (Montoro et al., 2010; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008). Therefore, we thought that trying this extract as a radio protective agent against ionizing radiation (IR) may prove beneficial effect (Maskey, Lee, Kim, & Kim, 2013). Since BC patients were exposed to IR, the genotoxic effects of IR were studied in white blood cells, which circulate throughout the body and which have been confirmed as a useful biodosimeters by many radiation studies (Lamkowski et al., 2014). The alkaline comet assay was selected as a sensitive method for detecting DNA damage produced by known or potentially genotoxic substances (Garaj-Vrhovac et al., 2002).

The current study demonstrated that radiotherapy is accompanied by significant increased levels of DNA damage in peripheral blood leukocyte of BC patients. It was not surprising because IR causes a wide variety of DNA damage ranging from single and double strand breaks in DNA as well as DNA base modifications, oxidative damage and alkali-labile lesions that can be easily converted into strand breaks during alkaline denaturation and therefore sensitively detected (Little, 2000; Singh, 2000). It is known that, besides direct ionization of DNA, IR also causes an indirect ionization when free radicals formed as a result of the ionization of water molecules which damage the DNA (Gamulin, Garaj-Vrhovac, & Kopjar, 2007).

To avoid the harmful effect of radiations, propolis was given to chemotherapy received BC patients 10 days before radiotherapy during the period of receiving radiotherapy and 10 days after completing radiotherapy. The present study demonstrated the ability of propolis to reduce significantly the radiation induced DNA damage in peripheral white blood cells of BC patients received chemotherapy and exposed to IR. This result is expected since propolis has the capability to stimulate DNA repair mechanisms which in turn counteract the damage inflicted by IR (Gamulin et al., 2007). The protective effect of propolis against IR could also be explained by both the direct scavenging of free radicals produced by the indirect effect (Oršolić et al., 2007) and the activation of oxidative repair enzymes (Ramos & Miranda, 2007). Both scavenger and antioxidant properties are involved in the protection against induction of DNA damage by IR. This result suggests that propolis may possess promising radioprotective effect and may offer a quite measurable protection against DNA damage caused by IR as detected by alkaline Comet assay.

A prerequisite for a multicellular organism to survive is the ability to correctly replicate and repair DNA while minimizing the number of heritable mutations. To achieve this, cells need a balanced supply of deoxyribonucleoside triphosphate (dNTPs) the precursors for DNA synthesis. The rate limiting step is de novo biosynthesis of dNTPs is catalyzed by the enzyme ribonucleotide reductase (Hakansson, Hofer, & Thelander, 2006). The classic eukaryotic RNR enzyme consists of a large RRM1 and a small subunit RRM2. The RRM1 subunit contains the ribonucleotide binding sites and allosteric effector sites. The RRM2 subunit contains a non heme iron complexed with a tyrosyl free radical and is essential for catalytic activity. A somewhat unusual feature of the RNR enzyme is that it catalyzes a reaction that proceeds via a free radical mechanism of action, any agents that scavenge the tyrosyl radical thereby destabilizing the iron center of RRM2 with a resulting loss of RR enzymatic activity (Barker et al., 2006).

In the present study, it was found that in group of patients exposed to radiotherapy only RRM2 levels before and after completing radiotherapy showed significant difference and significantly higher than control values, on following those patients up to 30 months 44% of them became metastatic with shorter disease free survival (DFS) time (23 months). This result suggests that RRM2 may possess oncogene like properties and plays a potential role in tumor malignancy and metastasis. This hypothesis is supported by many various studies. It has been found that overexpression of RRM2 increased the malignancy of H ras transformed fibroblast and enhance the invasive potential of human cancer cells (Zhou et al., 1998).

Recently various studies have demonstrated that RRM2 plays additional roles in determining the malignant potential of tumor cells. For example: elevated expression of RRM2 has been found to increase the drug resistant properties of cancer cells and significantly enhance the invasive potential of many human cancer cells (Gautam, Li, & Bepler, 2003; Zhang et al., 2011) whereas knockdown of RRM2 results in the reversal of drug resistance and suppressed tumor growth, and decreased metastatic potential. Therefore, the upregulation of RRM2 after completing radiotherapy could indicate that RRM2 is related to the proliferation of cells and may be a malignancy determinant critically in mechanisms controlling tumor progression thus delineating the regulatory mechanisms of RRM2 is very important for understanding the control of cell proliferation and cancer and for designing better cancer therapeutics by targeting RRM2.

The supplementation of propolis 10 days pre, during and 10 days after completing radiation therapy caused a significant down-regulation of RRM2 level and became within the normal control level. Also it was noticed that after 30 months only 16% of these patients became metastatic with longer median DFS time (27 months) in comparison to patients received radiotherapy only (23 months). Although a number of agents have been developed as RR inhibitors, hydroxyurea has received the most attention in preclinical and clinical studies. Hydroxyurea achieved only limited success in clinical trials. Propolis is an effective iron chelator and disturbs the di-ferric iron center that stabilizes the tyrosyl free radical, critical for catalytic function of RRM2 small subunits (Ebrahimzadeh, Pourmorad, & Bekhradnia, 2008; Gülçin, Bursal, Şehitoğlu, Bilsel, & Gören, 2010). Through this effect, propolis may potently interfere with RNR function and ultimately enhancing radiosensitivity of breast cancer cells. This suggestion is consistent with that reported by (Hillman et al., 2004) who stated that the combination of genisten, one of propolis component, and radiation enhances radiosensitivity in human esophageal and cervical cancer cells and exerted inhibitory effect on DNA synthesis, cell growth and colony formation.

The mechanisms of radio-sensitization induced by RNR inhibitor have been assumed to involve cell cycle synchronization and/or inhibition of DNA repair. Propolis supplementation results in an accumulation of cells in G1, and preferentially kills cell in S phase (Sawicka, Car, Borawska, & Niklinski, 2012). An apparent radio-sensitization may occur because cells in S phase are more radio-resistant than those in G1. The result of the present study suggests the beneficial effect of propolis in cancer radiotherapy.

In the present study, TAC in serum of patients received radiotherapy only either before or after completing radiotherapy was significantly lower than in normal control group. Radiotherapy treatment leads to significant decrease in serum TAC. Similar to our study, other researchers reported a significant decrease of serum TAC after treatment of children with malignancy, with standard chemotherapy which was associated with increased ROS (Papageorgiou et al., 2005). The reduction in TAC could reflect consumption of endogenous antioxidants by free radical generation by disease process or treatment itself. Insufficient dietary intake of exogenous antioxidants during the treatment period might have also contributed.

The present biochemical study revealed that lipid peroxidation in the form of malondialdehyde (MDA) which is a good indicator of the degree of membrane lipid peroxidation, was significantly higher in BC patients than in normal control group and significantly increased to comparable extent after completing radiotherapy as compared to pre radiotherapy, indicating the presence of radiation induced oxidative damage. Our reasonable explanation is that, IR causes hydrolysis of water generating hydroxyl radicals ($OH^{\cdot}$). $OH^{\cdot}$ is considered as the most damaging of all free radicals generated in organisms and reacts readily with most cellular components including organic molecules (Jagetia, Rajanikant, Rao, & Baliga, 2003). Its reaction with polyunsaturated fatty acids of membrane phospholipids and with lipid hydroperoxides are characterized by extremely high rate constants and produce a variety of compounds such as malondialdeliyde, 4 hydroxy non enol and acrolin (Gutteridge, 1995) that may exert more toxic effects on cells including alterations in the structure and function of cell organelles (Rosen, Vinter, & Goldberg, 1989). In light of these results, the reduction in TAC and elevation in lipid peroxidation suggested an increased level of oxidative stress in BC patients received radiotherapy.

In the current study, it was observed that propolis administration plus radiotherapy significantly lowered the radiation induced lipid peroxidation in terms of MDA production and led to a significant increase in TAC capacity. On the basis of the results of this study, it is clear that propolis has comparable reducing power and antioxidant activity, since it is composed of nutritionally valuable substances and contains considerable amounts of polyphenols substance which may act as potent antioxidant.

Concerning the toxic effect of ionizing radiation on hematological parameters, the present study displayed that radiotherapy resulting in a highly significant decrease in the number of WBCs, platelets and significant fall in Hb concentration, which may be due to alternation in bone marrow as well as hemopoietic system of BC patients group. Similar observations were obtained by (Osman & Hamza, 2013).

The decrease in the values of hematological parameters may be assigned to direct damage caused by IR (Heda & Bhatia, 1986). The cellular elements of the blood are particularly sensitive to oxidative stress because their plasma membranes contain a high percentage of polyunsaturated fatty acids (PUFA) (Chew & Park, 2004). Therefore, the decrease in WBCs count might be the consequence of radiation induced lipid peroxidation and damage of their cell membranes or may be attributed to mitotic inhibition of the bone marrow precursors and/or direct destruction of mature circulation cells (Ramadan, 2007). The observed decline in the total leukocytic count was in agreement with (Abdelhalim & Moussa, 2013). The decrease in Hb concentration was due to changes in erythrocyte membrane emphasizes the formation of free radicals. The effect of free radicals on erythrocyte membrane may contribute to the eventual leak of hemoglobin out of the cells (Hussien, Darwish, & Ali, 2007).

On the other hand, group of patients received propolis plus radiotherapy showed a significant improvement of WBCs count, Hb concentration and platelets count and significant increase in serum iron. Propolis induced extensive proliferation of hematopoietic cells in the spleen and bone marrow (Oršolić & Bašić, 2005). Moreover, it improves the digestive utilization of iron and increases the regeneration efficiency of hemoglobin especially during recovery from an anemic syndrome (Haro et al., 2000). In addition, the high content of flavonoids in propolis improves and accelerates the generation of erythrocyte and hemoglobin (Harrison, Shasha, White, & Ramdeen, 2000). Moreover, the administration of propolis was found to modulate the peripheral blood mononuclear cells (PBMCs) mitogenic responses which was suggested to be due to the presence of immunoregulatory components (Harish, Rubinstein, Golodner, Elmaliah, & Mizrachi, 1996). The results obtained from this study highlight the possible protective role of propolis against radiation-induced damage to the hematopoietic system and may help to prevent anemia.

5. Conclusions

- Supplementation of propolis with radiotherapy treatment offer a quite measurable protection against DNA damage caused by ionizing radiation in patients leukocytes.
- It seems reasonable to predict that propolis may potently interfere with RNR function and ultimately enhancing radio-sensitivity of breast cancer cells.
- These results nominate propolis to be a good agent to attenuate radiation induced lipid peroxidation and hematological damage.
- This study may lay a foundation for the potential future use of propolis in combination with radiotherapy to protect normal cells from radiation induced oxidative stress.
- Larger prospective studies are required to confirm our findings.

Conflict of interest

No conflict of interest is declared.

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